

IV. SUPPORT FUNCTION: CONDENSATE PREPARATION

- A. Objective: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.
- B. Results: Seventy eight smokings of 17 different cigarette codes were performed to yield condensate for various chemical and biological assays.
- C. References:

Hellams, R. D. and McGee, N. H. Notebook No. 8613, pp.83-84

PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: R. D. Kinser
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PERIOD COVERED: June, 1988

I. TSNA REMOVAL STUDIES

- A. Objective: To explore the possibility of extracting TSNA from stored tobacco using an extraction fluid compatible with current processing.
- B. Results: The extraction of TSNA and alkaloids from filler along with removal of the same constituents from a recirculating extraction solvent stream by Amberlyst-15 cation exchange resin was examined this month. Solvent composition, temperature, flow rate, solvent multiples, and ratio of resin to filler weight were variables. HPLC and GC/TEA methods were used to analyze for alkaloids and TSNA respectively.

Increasing the ratio of resin to filler increased the removal of alkaloid and TSNA. Nicotine and TSNA were extracted almost quantitatively. Minor alkaloids were more difficult to remove. Increasing the percentage of ethanol to hexane and the solvent multiple (total volume) also increased alkaloid and TSNA removal.

Analysis of Cherry Red filler, which is high in minor alkaloids, required modification of the alkaloid analysis procedure. Methanol extraction was replaced by extraction with benzene/chloroform containing aqueous base.

- C. Plans: Continue the study of the effect of extraction variables on the procedure to minimize the % dry weight loss and enhance minor alkaloid removal. Tests will also be performed to determine if the spent resin can be regenerated for future use.

D. References:

Warfield, A. H. Notebook No. 8558, p. 144
Lambert, E. A. Notebook No. 8523, p. 155

II. CIGARETTE CONSTRUCTION PARAMETERS

- A. Objective: To study the effect of cigarette construction parameters on TSNA in smoke.
- B. Results: Cigarettes have been obtained which will enable the study of the effects of circumference, paper porosity, paper citrate level, packing density, and filler cut width on TSNA in smoke. Initial studies of 17 mm and 25 mm circumference cigarettes showed no differences in delivery of TSNA into smoke as a function of the amount of filler consumed during smoking.

C. Plans: To continue the investigation of the effect of the other variables on TSNA in smoke.

D. References:

Lambert, E. A. Notebook No. 8523, p. 156

III. TSNA PRECURSORS

A. Objective: To determine the precursors of MS TSNA.

B. Results: A study of the ability of organic nitrites to act as nitrosating agents was conducted using t-butyl nitrite mixed with dihexylamine and anatabine in acetonitrile. Formation of nitrosamines was monitored over several days and the results clearly showed that t-butyl nitrite is an effective nitrosating agent.

Addition of nitrate and anabasine to bright and oriental fillers illustrated that nitrosoanabasine was formed in large quantities. Also nitrate additions to bright and oriental at levels approximating those of burley filler did not produce increases in TSNA to equal the TSNA delivery of burley smoke. These studies were performed in order to test the effects of increased TSNA precursor addition on pyrosynthetic TSNA production and in the case involving oriental filler to determine if a natural inhibitor of TSNA production exists. These results indicate that nitrate is an effective agent for production of nitrosating agents during smoking, but that nitrate alone does not control the final TSNA levels in smoke. This indicates that other factors may be operative in the production of TSNA in addition to the presence of the alkaloid and nitrosating agent precursors. Inter- and intracellular separation of precursors and, in the case of oriental, pyrosynthetic inhibitors may be important factors. Also with oriental filler the addition of large amounts of added alkaloid to the filler may deplete the active inhibiting agent, thus giving the appearance that a natural inhibitor does not exist.

The preparation of the document on nitrosation precursors and reaction mechanisms relating to TSNA pyrosynthesis is in the final editing stages. Recommendations for future studies of nitrosating agents, nitrosation mechanisms, and methods of inhibiting pyrosynthesis are included. Implementation of some of the recommendations has already begun.

C. Plans: Continue implementation of the studies recommended in the nitrosation report. Use oriental CEL fractions mixed with alkaloids and nitrite to further investigate the possibility that an inhibitor to TSNA production is present in oriental tobacco.

D. References:

Haut, S. A. Notebook No. 8595, p. 88

Tickle, M. H. Notebook No. 8587, p. 163

Morgan, W. R. Notebook No. 8579, p. 46